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Protein Labeling of the Cryptorchid Testis of the Rat

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PROTEIN LABELING OF THE CRYPTORCHID
TESTIS OF THE RAT



by
MANNFRED A. HOLLINGER

A Thesis Submitted to the Faculty of the Graduate
School of Loyola University in Partial
Fulfillment of the Requirements
for the Degree of
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LIFE

Mannfred A. Hollinger was born in Chicago, Illinois, on June 28, 1939. He attended Taft High School, Chicago, Illinois. In August, 1961 he received the degree of Bachelor of Science from North Park College, Chicago, Illinois.

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The author has been co-author of the following publications:

1. Davis, J.R., Firlit, C.F. and Hollinger, M.A. 1963
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2. Davis, J.R., Morris, R.N. and Hollinger, M.A. 1964
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Testis Slices. Proc. Am. Assoc. Cancer Res., 5: 13.
3. Davis, J.R., Morris, R.N. and Hollinger, M.A. 1964
Incorporation of L-lysine-U-C¹⁴ into Proteins of Cryptorchid Testis Slices. Am. J. Physio., 207: 50-54.

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TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION	1
A. Testicular Descent	1
1. Phylogenetic relationship of testicular position	1
2. The mechanism of testicular descent	2
3. The necessity for testicular descent	3
4. Failure of the testes to descend	3
B. The Effect of Temperature on the Testis	4
1. Histological and morphological changes	4
2. The effect of local applications of heat to the testis	4
3. The effect of increased body temperature on the testis	6
4. Theories as to the cause of testicular atrophy	6
C. Testicular Metabolism	7
1. Normal testis	7
a. Carbohydrates	8
b. Proteins	9
c. Androgen production	12
2. Cryptorchid Testis	12
a. Carbohydrates	12
b. Proteins	14

c. Androgen production	17
D. Effect of Temperature on Kinetics of Metabolic Reactions	19
1. Optimum temperature	19
2. Complex systems	19
3. Heat activation	20
II. MATERIALS AND METHODS	21
A. Animals	21
B. Tracer	21
C. Preparation of the Tissues	21
D. Incubation of the Tissues	22
E. Extraction of the Protein	23
1. Solvents	23
2. Homogenization and centrifugation	23
3. Dessication	23
4. Preparation of protein plates	23
5. Measurement of radioactivity	24
F. Computation	24
G. Colorimetric Determination of Protein Content in Whole Homogenates and 15,000xG Supernatants	24
H. Preparation of Cryptorchid Animals	25
I. Glutamic Acid and CO ₂ Measurements	25
J. Measurement of Rectal, Peritoneal and Scrotal Temperatures	26

III. RESULTS	27
A. In Vivo Temperature Measurements	27
B. Kinetics of L-lysine-U- C^{14} Incorporation into Protein of Rat Testis Slices	27
C. Tracer Uptake in Rat Testis, Liver, Kidney, and Spleen	27
D. Effect of Temperature on Lysine Uptake	28
E. Viability of Rat Testis Slices	28
F. Effect of Abdominal Transplantation on Testi- cular Weight and Incorporation of L-lysine-U- C^{14} into Protein	29
1. Testicular weight	29
2. Lysine incorporation	29
G. Effect of Age and Abdominal Confinement on the Specific Activity of Rat Testis Slices	30
1. Effect of age on specific activity	30
2. Abdominal confinement	30
H. Effect of Temperature on the Uptake of Labeled Lysine in the Adult, Scrotal Testis, the Cryptorchid Testis, Immature Testis, and an Androgen-producing Interstitial Tumor	31
I. Histological Comparison of Scrotal Testis, Cryptorchid Testis, Immature Testis, and Interstitial-cell Tumor	31
1. Adult scrotal testis	31
2. Cryptorchid testis	31
3. Immature testis	32
4. Androgen-producing interstitial-cell testicular tumor	32

J.	Protein Content of 15,000xG Supernatants of Scrotal and Cryptorchid Testes Utilizing the Lowry Method	33
K.	Protein Content in Whole Homogenates of Host Scrotal and Cryptorchid Testes Utilizing the Lowry Method	33
L.	Moisture Content of Host Scrotal and Cryptorchid Rat Testis	34
IV.	DISCUSSION	35
V.	SUMMARY	44
VI.	FIGURES	47
Figure I	In Vivo Temperature Measurements	48
Figure II	Effect of Time of Incubation on Lysine Incorporation	49
Figure III	Incubation Apparatus	50
Figure IV	Effect of Temperature on Incorporation of L-lysine-U- C^{14} into Protein of Rat Tissues	51
Figure V	Effect of Time and Temperature on Lysine Uptake	52
Figure VI	Effect of Temperature on Testis Slices Viability	53
Figure VII	Effect of Abdominal Transplantation on Testicular Weight and Lysine Uptake	54
Figure VIII	Effect of Animal Age and Lengths of Abdominal Confinement on Specific Activity	55
Figure IX	Effect of Temperature on Lysine Incorporation into Various Testicular States	56

Figure X	Histological Comparison of Adult, Scrotal Testis, 20-day Cryptorchid Testis, Immature Testis, and Inter- stitial-cell Tumor	57
VII. TABLES		58
Table I	Protein Measurements in 15,000xG Super- natant Fractions	59
Table II	Protein Contents of Whole Homogenates . .	60
Table III	Moisture Content Determinations	61
BIBLIOGRAPHY		62

CHAPTER I

INTRODUCTION

A. TESTICULAR DESCENT

The incidence of cryptorchidism in man is 10 per cent at birth, 2 per cent at puberty, and 0.2 per cent at maturity (86). Charny concurs when he says, "Two of every 1,000 men in the United States are sterile due to bilateral cryptorchidism." (14). Although the percentage of afflicted individuals is relatively low, the absolute number of cases is large enough to make it a not uncommon clinical experience for the physician. The cryptorchid male suffers not only from possible sterility, but also from the associated stigma of an empty scrotum. Finally, an increased incidence of testicular tumors has been reported in cryptorchid testes, therefore becoming a serious complication (13). Zimel (87) ascribes the higher incidence of neoplasia to the tropic influence of the pituitary.

1. Phylogenetic relationship of testicular position.

The descent of the testes is a trait unique to mammals. In all classes, up to and including the Reptilia and Aves, the testes occupy a position high in the abdominal cavity. This position persists in such primitive mammals as the Monotremata (duck-billed platypus and the spiny anteater). The earliest extra-abdominal position, according to Andrews and Bissel (1),

occurs in the Marsupials (kangaroo), where the testes lie in the supra-pubic region in a pouch. In some rodents (ground squirrel) and some of the Insectivora (mole) the testes are intra-abdominal in the resting phase of the sexual cycle. During the mating season they are extruded. Thus, cryptorchidism is normal for some animals.

2. The mechanism of testicular descent.

It has been generally agreed that the gonadotrophic hormones play a role in providing the stimulus for testicular descent. Rost (67) found that the descent of testes was inhibited in hypophysectomized rodents. Engle (24) has reported that from the seventh week to the ninth month chorionic gonadotrophin is present in high concentration in the maternal circulation, which is concomitant with the normal period of testicular descent. Martins (49) in an interesting experiment found that if he substituted a piece of paraffin for an inguinal testis it could be stimulated to descent by administration of chorionic gonadotrophin.

The presence of an intrinsic factor in the developing testis which controls descent is a possibility. Animals such as the woodchuck and the hedgehog withdraw their testes into the abdominal cavity during the winter and re-descend them during the rutting season. During the period of abdominal confinement the germinal epithelium degenerates, but upon descent it regenerates (14). These observations infer that the testis has an inherent ability to respond to changes in season or to variations in

gonadotrophin secretion.

3. The necessity for testicular descent.

Although postulates have been presented in regards to the mechanism of descent the question of why the event takes place at all remains a curious query. The concensus, in general terms, is that the migration is a search for lower temperature. In 1922 Crew (18) first drew attention to the scrotum as a temperature regulating device.

The scrotum is a pouch of very thin skin amply supplied with subaceous glands (apparently lacking in man) and responds quickly to alterations in temperature. In a warm environment the scrotum becomes flaccid, while in cooler confines it closely surrounds the testes and draws them against the body wall. In conditions of scrotal thickening such as elephantiasis where the scrotum becomes incapable of temperature regulation, the testes atrophy.

4. Failure of the testicles to descend.

Two major premises have been put forth to explain the etiology of cryptorchidism. The first is based on mechanical abnormalities which interfere with the transport of an otherwise normal testis. This includes such things as peritoneal adhesions, short vas deferens, and obstruction to the passage caused by abnormal intrauterine position of the fetus. The second proposes a testicular deficiency which can be either primary or secondary in nature. Primary failures refer to a lack of impetus to descend while secondary is concerned with the failure of the testis to

respond to the normal impetus.

B. THE EFFECT OF TEMPERATURE ON THE TESTIS

1. Histological and morphological changes.

Animals whose testes are normally scrotal require their descent for normal spermatogenesis to occur. Failure to descend causes testicular atrophy and interference with the normal spermatogenic processes (28, 55). The destruction of the germinal epithelium of the testis when placed in the abdominal cavity has long been attributed to an increased environmental temperature (18, 58). Bascom and Osterud (6) measured the length and diameter of seminiferous tubules in both the normal and cryptorchid testes. The cryptorchid testis was found to have a total tubule length equal to that of the normal testis while the diameter was reduced in size. The more mature germinal cells of the testis appear to be the most susceptible to deterioration when subjected to an abdominal environment (60). The spermatogonia, Sertoli cells and the interstitial tissues are much more resistant and seem to be relatively unaffected by artificial cryptorchidism (16, 60, 83). Korenchevsky (38) observed that bilateral cryptorchidism did not significantly affect the total body weights of rats, but there was an increase in the deposition of body fat.

2. The effect of local applications of heat to the testes.

Fukui (27) found, in 1923, that if heat was applied to the testes, in various forms, degeneration of the seminiferous tubules followed. He also found that if both testes were elevated into

the abdominal cavity and one external side was artificially cooled, that upon examination of the testes within a few days the testis from the cooled side appeared normal, while the other one showed degeneration of the tubules. Moore and Oslund (54) found that by insulating the scrotum of Rams with an encasing woolen garment that after eighty days the testes were devoid of spermatozoa and in effect, "the animal had sterilized itself with its own body heat."

Subsequent investigations by Moore and Quick (55) on rats, rabbits, and guinea pigs found the temperature of the scrotum to be appreciably lower than the peritoneal temperature. In most animals the difference is a nature of 1 to 8.5°C. In the case of the white rat, the difference between the peritoneal cavity and the scrotal temperature has been shown to range from 4.0 to 7.4°C depending on ambient temperature (53). In man, the difference is approximately 1.5 to 2.5°C (86).

Moore and Quick (55) found that if hot water pads were applied to the scrotum of guinea pigs an elevation in temperature of some 7 to 8°C was obtained. Examination of the testes revealed severe tubule degeneration following a ten day recovery period. Cunningham and Osborn (19) were able to sterilize rats in 5 minutes with infra-red treatments at 48°C. The rats became temporarily infertile for one month following a 54 day latent period. Moore (59) found that a ten minute exposure of the scrotum of a guinea pig to water warmed 6°C above body temperature results in

great damage to the tubules.

3. The effect of increased body temperature on the testis.

The deleterious effects of increased temperature on the testes are not restricted to local applications. Macleod and Hotchkiss (43) showed that if the body temperature was experimentally increased, there was a reduction in the concentration of spermatozoa in the ejaculated semen. Clinical conditions associated with pyrexia such as pneumonia and typhoid have been implicated in this respect (44). Macleod (44) has also shown that chicken pox can cause temporary interference with sperm production.

4. Theories as to the cause of testicular atrophy.

The fact that temperature has a profound influence on the testes is beyond refute. However, the specific execution of this effect is as yet unknown. Fukui (27) has suggested that the testicular damage may be a result of destruction of certain thermolabile "spermatogenous" proteins. Barron (5) indicated that "heat degeneration" may be due to hyperemia caused by local vasodilatation. Moore (54) believes vascular stagnation and oxygen lack to be the cause. Gross, however, reports that oxygen tension in the testis of rabbits increases when the scrotum is warmed (29). Young (85) reports that varicocoele brings about a significant increase in sub-fertility in many patients. He postulates that this may be due to either an interference with the nutrition of the germinal epithelium because of venous congestion or of increase in testicular temperature. Interference with the

testicular blood supply for as little as 2-3 hours may cause irreversible damage (33). Oettle and Harrison (64) found that prolonged ischemia resulted in atrophy of the tubules, disappearance of the lumen, and the Sertoli cells became embedded in a collagenous matrix. Bernstorff (9) found that sectioning of the testicular artery resulted in atrophy of the testis. In the surviving tubules the primary spermatocyte was usually the most advanced type of germinal epithelial cell.

Harrison and Weiner (31) and Dale and Herrich (20) propose that thermal exchanges occur between the arterial and venous blood, which in favorable situations could pre-cool the testicular inflow. The authors showed the temperature of the testicular arterial blood in the anesthetized dog to be approximately 3°C lower than that of the aortic blood. Waites and Moule (79) found that blood in the spermatic artery of the ram was approximately 5°C lower than that of the aorta when testicular temperatures were maintained between 33 and 34°C. These results suggest a temperature regulating function for the testicular venous system, an interference with which could result in an elevated intratesticular temperature. Although it is known that changes in circulation do affect spermatogenesis, its relationship to the germinal degeneration in the cryptorchid testis remains unresolved.

C. TESTICULAR METABOLISM

1. Normal testis.

a. Carbohydrates

The metabolism of the testis has been found to be similar to that of the brain in that they both show high anaerobic glycolysis (81), and their respiration is very dependent on the presence of glucose or other substrate (22). Warburg (80) has shown, however, that the testis differs from brain and other tissues in three respects: (1) the testis shows a relatively high aerobic glycolysis which is not inhibited by adding lactate; (2) the testis carries out a considerable metabolism of pyruvate under anaerobic conditions; (3) the testis forms, in anaerobic experiments without glucose, a considerable amount of acid which is not lactic acid. The testis was found by Benoy and Elliott (8) to lack the ability to synthesize glucose from lactate or pyruvate. Elliott et al. (23) found lactate to be readily oxidized by the testis to pyruvate, which in turn is rapidly metabolized. Fumarate and malate were also found to be oxidized by testis tissue as measured by O_2 uptake. Acetate does not appear to be an essential intermediate in the oxidation of pyruvate since the oxidation of pyruvate proceeds in the absence of glucose while that of acetate does not. Annison et al. (2) studied the comparative oxidative metabolism of glucose and acetate in ram testis. They found glucose to be a more important metabolite than acetate; glucose exhibited greater A-V difference thus suggesting preferential removal of this substrate from the blood.

Tepperman et al., studied the metabolism of the rat testis in vitro (76). They found that as the age of the animal increases, there is a decrease in the QO_2 . This progressive decrease was alleviated by the addition of exogenous glucose with the result that there was no significant difference in QO_2 in the age groups studied. Respiratory quotient measurements of adult and immature testes revealed no significant difference. These investigators also studied the aerobic glycolysis of adult and immature testes. The results demonstrated a comparatively higher aerobic glycolysis in the immature testis. Later work by Tepperman and Tepperman (76) revealed that adult testes are able to oxidize glucose added to the incubation medium.

Arai (4) reports that if fumaric acid is administered chronically to rabbits, testicular atrophy results. He suggests that some sulfhydryl containing enzyme(s) may be adversely affected, since the results can be reversed by the addition of cysteine.

b. Proteins

Although speculation concerning the importance of testicular proteins was made as early as 1923 (27) it has been only relatively recent that investigators have begun to explore the relationship of these proteins (enzymes) to the function of the organ.

Niemi and Ikonen (62) in 1962 studied the cytochemistry of oxidative enzyme systems in rat Leydig cells in order to determine

their functional significance. These investigators found that the rat Leydig cells exhibited activities of NAD and NADP diaphorase and succinic, lactic, glutamic, α -glycerophosphate, β -hydroxybutyrate and steroid dehydrogenases. They were able to correlate the intensity of hypophyseal stimulation with the activities of β -hydroxybutyrate and steroid-3 β -ol-dehydrogenase in the interstitial cells. The other enzymes appeared to be non-responsive. They suggested that these enzymes might be used as indicators of the level of testosterone production. Schor and his co-workers (71) have shown that chorionic gonadotrophin stimulates respiratory enzymes (isocitric dehydrogenase) and enzymes belonging to the hexose monophosphate shunt (glucose-6-phosphate dehydrogenase). They postulate that this mechanism plays a role during the in-utero development of the testis. Pituitary gonadotrophins may provide the stimulus after parturition. Hall (30) has shown that ICSH stimulates the incorporation of valine-1-C¹⁴ and tryptophane-1-C¹⁴ into protein of rabbit testis slices in vivo or in vitro. Thyroxin has been found to have no effect on the incorporation of L-leucine-C¹⁴ into rat testicular protein (52).

Bhargava (10) found that washed spermatozoa and ejaculated sperm from the bull, known to contain only traces of ribonucleic acid (RNA), were able to incorporate C¹⁴ labeled amino acids into protein in vitro. Martin and Brachet contradicted these results by employing radioautography to assay labeled amino acid uptake.

Their findings were that the radioactive tracer was localized in cell debris rather than in the spermatozoa (48). These results rule out the proposed exception of bull spermatozoa as being able to carry out high rates of protein synthesis while possessing low RNA content.

c. Androgen production

The synthesis of hormones by the testis constitutes one of its two major functions, the other being the production of spermatozoa. The testis is known to secrete two hormonal types, androgens and estrogens (86). The synthesis of androgens has been unanimously ascribed to the Leydig cells. The testicular site of estrogen production, however, remains a controversy; either Sertoli (34) or Leydig cells (45) may be the source. In this consideration androgens will be of major interest.

The biosynthesis of androgens is mediated through the influence of Interstitial-cell Stimulating Hormone from the anterior lobe of the pituitary. This trophic hormone is concerned with the maintenance and repair of the Leydig cells. Its release is brought about by low levels of circulating androgens.

The prime importance of androgen synthesis is the control of secondary sexual characteristics. Castration of a male animal results in atrophy of the seminal vesicles and the prostate gland. Both organs can be restored to normal with the administration of testosterone (86). The exact biochemical mechanism of action of testosterone is as yet unknown although some

observations have been made. Rudolph and Samuels (68) report that testosterone reverses the depressed respiration of seminal vesicles in castrate rats. Lowered citric acid levels in castrates have been elevated by the use of testosterone (47). Fructose production by the seminal vesicle is stimulated by the addition of testosterone (46).

2. Cryptorchid testis.

a. Carbohydrates

Although the gross histological changes of the testis exposed to supra-normal scrotal temperatures has been known for some time, it is only recently that investigators have had the technical facilities to study the comparative biochemistry of the normal and cryptorchid testis. Tepperman (76), in a study on rat testis metabolism, reported that QO_2 values did not change over the age range studied if glucose was present in the incubation medium. If the substrate was not added, there was a progressive decrease in QO_2 as the animals became older. This suggests that as the testis becomes more mature, in terms of cellular components, the overall metabolism is decreased. The measurement of QO_2 in the cryptorchid testis, in the absence of substrate, revealed a significantly higher value than that of normal testis tissue. This would be expected since the more mature cell types are not present. The glucose respiratory quotient for the cryptorchid testis was determined as 0.5 as

compared with a value of 0.93 for normal tissue. This is not surprising in view of the failure of the cryptorchid testis to oxidize glucose (47). These results infer that the primordial germinal elements utilize some other substrate. No difference was found to exist between cryptorchid tissue and normal tissue regarding aerobic glycolysis.

Ewing and Vandemach (25) found that if rabbit testes were exposed to abdominal temperature for 24 hours, their tissue contained 12 per cent less glucose and 27 per cent less lactic acid than control tissue. If testes which were exposed to various abdominal-scrotal temperature gradients were assayed for glucose uptake, lactic acid production, and O_2 uptake, glucose uptake was stimulated, while lactic acid production and O_2 uptake were depressed as the temperature difference increased. Ewing and Vandemach (25) explain these results by suggesting that large amounts of glucose were removed from the tissue by the increased metabolic rate induced by exposure to the elevated abdominal temperature, and that the depleted supply was replaced from the exogenous source in the Warburg flask. Experiments were also carried out in which normal and 24 hour cryptorchid testis tissue was pre-incubated in cold glucose solution. The results obtained showed no depression of lactic acid production or O_2 uptake. These investigators conclude that testes exposed to the higher temperature of the abdomen undergo a transient increase in metabolism which is then followed by a decrease in metabolic

activity as the available endogenous substrate is exhausted. This study suggests that spermatogenic arrest which results from exposure of the testis to elevated temperatures may be related to reduced levels of substrate in the tissue. Since the time of abdominal confinement was only 24 hours, the metabolic effects observed were probably a reflection of alterations in tubular spermatozoa and spermatids in view of the fact that they are the first to be adversely affected by heat.

The exact significance of these metabolic alterations is as yet undetermined. They do illustrate, however, the influence of the various testicular cell types in reference to the composite metabolism of the organ. Because of the preponderance (90 per cent of the normal rat testis (39)) of the seminiferous tubules, testicular metabolism is largely determined by their presence. In cryptorchidism the germinal degeneration reduces their presence to 60-70 per cent (39) and this unmasks the metabolic pattern of the Leydig cells, Sertoli cells, and connective tissue cells to some extent. Tepperman (76) explains the elevated QO_2 of the cryptorchid testis in terms of a decreased germinal cell, Leydig cell ratio. Steinberger (74) concurs that the Leydig cells are the responsible cells, but adds that their influence is mediated through pituitary gonadotrophins.

b. Proteins

Studies concerning testicular protein have mainly concerned themselves with changes in various enzyme activities

and attempting to correlate these changes with observed differences in cryptorchid and normal testis metabolism.

Ford and Huggins (26) measured malic dehydrogenase and lactic dehydrogenase in normal and cryptorchid testes from adult rats. Increased concentrations over the controls were observed beginning 4 days post-operative. The levels reached their maximum at approximately 10 days and remained elevated throughout the 74 day observation period. These results indicate an elevated metabolic picture. Turpeinen et al. (78) exposed testes to warm water (44°C) for 20 minutes and found a change in the distribution of succinic dehydrogenase and acid phosphatase. The chief locus of their activity was shifted from the seminiferous epithelium to the interstitium. The authors suggested that this could represent a stimulation of oxidative metabolism in this tissue. That these changes do not represent the primary damaging effect of the elevated temperature is shown by the fact that there were no alterations in these enzymes 24 hours after heat treatment, while histological damage was demonstrable. Kormanó (39) likewise found an increased activity of succinic dehydrogenase in the cryptorchid testis and proposed this as evidence for an increased oxidative metabolism of the Leydig cells. Additional histochemical results revealed (39) a parallel loss of β -hydroxybutyrate and steroid dehydrogenase up to the 21st day following the cryptorchid operation. Normal levels of glucose-6-phosphate dehydrogenase were found in the Leydig cells of cryptorchid testes.

suggesting a normal production of NADPH by the hexose monophosphate shunt pathway. Normal lactic dehydrogenase activity indicated a normal glycolytic process, therefore affirming the normal glycolytic values of the cryptorchid testis as reported by Tepperman (76).

Hayashi, et al. (32) found the activities of β -glucuronidase, esterase, and lipase increased following experimental cryptorchidism. Huggins (35) reported that induced cryptorchidism caused an increase in esterase concentration while the total content of the testis decreased. Sakatoku (69) found that alkaline phosphatase activity of the tubules decreases after 14 days of abdominal confinement.

Steinberger and Nelson (72) in studying the effect of various factors on hyaluronidase concentration in the testis found a drop in concentration 4 days following cryptorchid surgery. Complete disappearance of the enzyme occurred by the 15th day. Turpeinen (78) observed a marked reduction of hyaluronidase 21 to 30 days after a 20 minute exposure to 44°C water. Steinberger and Nelson attribute the drop in hyaluronidase concentration to the absence of spermatids.

Llaurado and Dominquez (40) investigated the effect of unilateral cryptorchidism on the enzymes involved in androgen biosynthesis. They found that after three weeks exposure to the abdominal environment, the cryptorchid testis ability to synthesize testosterone was markedly reduced.

c. Androgen production

The literature concerning the effect of temperature on testicular endocrine function contains a wealth of controversy. One possible explanation might lie with the different sensitivities of the methods employed by the various investigators. Another possibility could be the heterogeneity of the animals studied. Young, in fact, has reported that the severity of endocrine alterations in cryptorchidism is species dependent (86). Moore (56) has demonstrated that the effect of cryptorchidism on androgen production is dependent on the age at which it is induced, older animals appearing less sensitive.

Secondary sex characteristics have been used by many investigators as indicators of androgen production. However, the sensitivity of this criterion is suggested by the fact that castration changes appear in the pituitary 75 days following transplantation of the testis, while the seminal vesicles and prostate do not become atrophic until 240 and 400 days, respectively (86). Moore (57) reported maintenance of secondary sex characteristics in the boar, bull and stallion following bilateral retention of the testes. Schlotthauer and Bollman (70) found that the prostate of a dog could be maintained for two years if the animal was rendered unilaterally cryptorchid and the remaining testis removed. Antliff (3) found no significant interference with endocrine activity, as measured by secondary sex characteristics, in a guinea pig rendered cryptorchid for three years. Weil (82), on

the other hand, found that if a guinea pig was made unilaterally cryptorchid the normal scrotal testis underwent stimulation of the seminiferous epithelium, thus inferring a great enough decrease in androgens to merit gonadotrophin release from the pituitary.

Johnson (36) took immature male rats and (1) either exposed them to X-irradiation, or (2) rendered them cryptorchid, or (3) subjected them to both. The animals were then placed in parabiosis with castrated males. Pairs were autopsied at various intervals. Androgen production in response to endogenous gonadotrophin from a castrated animal was better in irradiated than cryptorchid animals, indicating a decreased ability to synthesize androgens. Clegg (15) in experiments on the rat measured the fructose content of the seminal vesicle as an indicator of androgen production. He reported a transient increase followed by a decrease. A histological examination confirmed the findings.

Huggins (35) reported that in the rat testis, esterase is located in the interstitial cells and that its concentration is related to androgen production. In puberty the increase in esterase was concomitant with an increase in prostatic weight. Hypophysectomy caused a dramatic reduction in testicular esterase. The injection of gonadotrophins stimulated a return to almost normal levels. Cryptorchidism caused a reduction in the total content of testicular esterase.

Llaurado and Dominquez (40) reported that induced cryptorchidism resulted in failure of the organ to convert progesterone

to 17-hydroxyprogesterone, 4-androstenedione and testosterone.

Kormano's (39) finding that cryptorchidism resulted in decreased activity of β -hydroxybutyrate and steroid dehydrogenase, enzymes believed to play a role in androgen biosynthesis, suggested interference with steroid production.

Kimeldorf (37) reported a 40 per cent decrease in urinary 17-ketosteroid concentration in cryptorchid rabbits.

The findings of the more contemporary investigators appears to have demonstrated conclusively that in cryptorchidism there is an alteration in the biosynthetic pathway of testosterone and that this change is manifested by a decreased synthesis of androgen.

D. EFFECT OF TEMPERATURE ON KINETICS OF METABOLIC REACTIONS

1. Optimum temperature.

The optimum temperature of an enzyme is determined by the balance of the enzymatic reaction rate and the rate of destruction of the enzyme. In a multienzyme system, such as a slice system, there are many reactions proceeding simultaneously and the rates of these reactions will generally react to temperature in different ways. Temperature may also influence enzymatic reaction rates by altering diffusion processes of substrates.

2. Complex systems.

Because complex systems are more unstable than their constituents, proteins are more unstable at elevated temperatures than amino acids and cells even more so than the former two.

Most mammalian cells are adversely affected by increases in

temperature of 5°C above their physiological value. The difficulty in determining which specific system is altered is that all of the cellular activities are closely related. If a particular system is isolated, its naturally occurring relationship to the over-all metabolic scheme is broken. This is apparent in protein synthesis. For example, if the cellular respiratory apparatus is impaired in any way, resulting in decreased energy production, then this becomes a rate limiting factor in the synthesis of the proteins. Upon separate investigation, each isolated system may be found to operate normally, and yet the entire system is not operable, the delinquent entity being a co-factor such as a hormone.

3. Heat activation.

Another aspect should be mentioned at this point, and that is "heat activation" of enzymes (75). This phenomenon is concerned with enzymes that are normally bound to inhibitors, also of a protein nature, under physiological conditions. An increase in temperature destroys the inhibitor and liberates the previously inactive enzyme. The enzyme is then free to express itself in its own particular way.

The previous examples illustrate the various ways in which temperature can affect metabolism. It will not be the purpose of this study to investigate each of these factors, but rather to attempt to correlate the data obtained with suggested mechanisms.

CHAPTER II

MATERIALS AND METHODS

A. ANIMALS

The animals employed in these experiments were male Sprague-Dawley rats, 55 to 65 days of age and weighing 185 to 220 grams. They were obtained from the Abrams Small Stock Breeders, Chicago, Illinois. The animals were housed at a constant temperature of 72°F. Their diet consisted of Rockland laboratory chow eaten ad libitum.

B. TRACER

The tracer employed was L-lysine uniformly labeled with isotopic C^{14} . The L-lysine- $U-C^{14}$ used had a specific activity of 5.8 mc/mole and was purchased from the Nuclear Chicago Corporation.

C. PREPARATION OF THE TISSUES

The animals used were sacrificed by decapitation and taken into the cold room where the temperature was 4°C. The testes were removed and weighed to the nearest milligram on a Roller-Smith torsion balance. The testicular capsule was removed and slices made with the aid of a Stadie-Riggs microtome. The same procedure was followed when other organs were used. While the excisement and slicing of the tissues was being carried out, previously prepared Warburg flasks containing 3.0 ml of Krebs-Ringer bicarbonate buffer at pH 7.4 in the main chamber and 0.2 ml of L-

lysine-U-C¹⁴ in the side arm (21) were equilibrating to the temperature of the cold room. The Krebs-Ringer bicarbonate buffer had been pre-gassed with 95 per cent O₂ and 5 per cent CO₂ for ten minutes. To each Warburg flask 150 to 200 mg of tissue was added and swirled. The flasks were then quickly taken and placed on the manometers.

D. INCUBATION OF THE TISSUES

The flasks were gassed for ten minutes with 95 per cent O₂ and 5 per cent CO₂ while adjusting to the temperature of the bath. Following the equilibration period, the labeled lysine was added and the incubation carried out for the desired time at 140 oscillations/minute. The final concentration of L-lysine-U-C¹⁴ in the incubation flask was 1.8×10^{-5} M. Incubation of tissue slices was carried out simultaneously at temperatures ranging from 20 to 44°C. This was accomplished by using several temperature-controlled water baths surrounding a main Warburg apparatus (Figure 3). The incubation period was terminated by dumping the contents of the Warburg flask into a test tube containing 0.3 ml of 5N perchloric acid. The test tubes were then allowed to stand under an exhaust hood for 15 minutes. During this period, occasional mixing on a Vortex Jr. mixer ensured complete denaturation. The samples were then centrifuged at 600xG for 10 minutes and the supernatants discarded.

E. EXTRACTION OF PROTEIN

1. Solvents.

The extraction procedure was then carried out with the addition of 0.6N perchloric acid (to complete the denaturation), cold 5 per cent trichloroacetic acid (TCA), hot 5 per cent TCA for ten minutes at 87°C (for removal of nucleic acids), 95 per cent ethanol, 100 per cent ethanol, 2:1 chloroform:methanol (for removal of fats), benzene, and finally two times with anhydrous ethyl ether (12).

2. Homogenization and Centrifugation.

After the addition of each solvent the sample was then homogenized with either a motor driven teflon pestle or a Vortex Jr. mixer. The sample was then centrifuged at 600xG for ten minutes and the supernatant discarded.

3. Dessication.

Following the extraction with ethyl ether the tubes were initially dried in a dessicator which was evacuated with a suction apparatus attached to a tap outlet. Complete dryness was obtained by allowing the tubes to stand at room temperature overnight. The tubes were always covered with filter paper, held on by rubber bands, to prevent any foreign material from falling into them.

4. Preparation of protein plates.

The resulting fine white powder was then added with a spatula into the center-well of a stainless steel planchot. The protein was then compressed into as thin a layer as possible by placing a

stainless steel pestle over the powder and applying one sharp blow with a hammer.

5. Measurement of radioactivity.

The plates were then taken and assayed for radioactivity in a Nuclear-Chicago apparatus consisting of a Model C-110B Automatic Sample Changer, a Model 183 Scaling Unit, and a Model C-111B Printing timer.

F. COMPUTATION

The uptake of L-lysine- $U-C^{14}$ into tissue protein has been expressed both in terms of specific activity (counts per minute/mg dry weight protein) and as total counts per minute/100 mg wet weight tissue slice. In the latter case, the total weight of the protein from the various tissues were obtained by subtracting the weight of the tube from the weight of the tube containing the final dry powder. The total L-lysine- $U-C^{14}$ in protein of each tissue slice was then determined by multiplication of the specific activity of the tissue by the dry weight of protein in the tissue and this figure corrected to 100 mg wet weight of slice.

G. COLORIMETRIC DETERMINATION OF PROTEIN CONTENT IN WHOLE HOMOGENATES AND 15,000xG SUPERNATANTS

Protein measurements of whole homogenates and 15,000xG supernatant fractions were also made. The testes were homogenized in a Potter-Elvehjem glass homogenizer at 4°C in pH 7.5 Tris buffer (65). The 15,000xG supernatants (containing microsomes and pH 5 fraction) were obtained with an International Ultracentrifuge.

Model HR-1, at 12,000 rpm for thirty minutes at a temperature of 4°C. Aliquots were then taken and the protein content determined according to the method of Lowry (41).

H. PREPARATION OF CRYPTORCHID ANIMALS

The cryptorchid operations were performed on 60-day old animals. The cryptorchid animals were prepared, using ether anesthesia, by making a mid-line incision in the abdomen and gently forcing the right testis into the abdominal cavity by applying pressure to the bottom of the scrotal compartment. A fine (6-0) silk suture was then passed under the capsule of the testis and anchored to the lateral body musculature. In all cases extreme care was exercised not to traumatize the organ in any respect or to obstruct any vasculature. The left testis was allowed to remain in the scrotum and was used as the control. These testes were excised at various time points ranging from 4 to 40 days following abdominal transplantation for incubation. Animals were also sacrificed at different times and their testes removed for histological study.

I. GLUTAMIC ACID AND CO₂ MEASUREMENTS

The following procedure was employed for the determination of conversion of glucose to CO₂ and glutamic acid in slices of rat testes. The main chamber of the Warburg flask contained 200 mg of tissue in 3.0 ml of Krebs-Ringer bicarbonate buffer at pH 7.4. The sidearm contained 2.5x10⁵ counts/minute of D-glucose-U-C¹⁴ in a volume of 0.2 ml. The final concentration of radioactive

glucose in the incubation flask was $2.6 \times 10^{-6} \text{M}$. Total C^{14}O_2 production was determined as the barium carbonate (63). Radioactivity in glutamic acid was determined by ion-exchange chromatography of the perchloric acid-soluble supernatant using columns of Dowex-x8 resin eluted by an acetate gradient (11).

J. MEASUREMENT OF RECTAL, PERITONEAL AND SCROTAL TEMPERATURES

A Yellow Springs Tele-Thermometer with a temperature range of 0 to 50°C was utilized to obtain the rectal, peritoneal and scrotal temperature of the male white rat. A direct reading probe was inserted just cephalad of the anal sphincter for the rectal measurement. For the peritoneal temperature determination the probe was inserted to a point within the approximate vicinity of the liver. The scrotal temperature was measured by directly perforating the scrotal wall and introducing a hypodermic probe.

CHAPTER III

RESULTS

A. IN VIVO TEMPERATURE MEASUREMENTS

Measurements of rat rectal, peritoneal, and scrotal temperatures were made, at room temperature (Figure 1). It can be seen that while there is virtually no difference in the temperature of the rectum and peritoneum, there does exist an approximate 3°C decrease in temperature from the abdominal cavity to the scrotal compartment.

B. KINETICS OF L-LYSINE-U-C¹⁴ INCORPORATION INTO PROTEIN OF RAT TESTIS SLICES

The time course of the incorporation of L-lysine-U-C¹⁴ into protein of rat testis slices incubated at 37.5°C is presented in Figure 2. A progressive increase in protein labeling occurred from 0 to 60 minutes with increased incorporation of the tracer still present up to 120 minutes. From these data, as well as similar experiments employing slices of liver, kidney, and spleen incubated at 37.5°C, a one-hour incubation period was chosen as a convenient time for investigating the effect of temperature on protein labeling in these tissues.

C. TRACER UPTAKE IN RAT TESTIS, LIVER, KIDNEY AND SPLEEN

Figure 4 indicates the effect of temperature on the incorporation of radioactive lysine into protein of slices of rat

testis, liver, kidney, and spleen. In each case, the time of incubation with the tracer was one hour and the gas phase was 95 per cent O_2 and 5 per cent CO_2 . In the testis, maximal incorporation of L-lysine- $U-C^{14}$ into protein occurred at $32^\circ C$. In the liver, kidney, and the spleen, however, the amount of isotope incorporated into protein increased uniformly from 20 to $37.5^\circ C$. Moreover, upon incubation of slices of liver, kidney, and spleen at $44^\circ C$, essentially no radioactivity was found in protein, indicating that the maximal incorporation of labeled lysine into protein of these tissues occurred between 37.5 and $44^\circ C$.

D. EFFECT OF TEMPERATURE ON LYSINE UPTAKE

The incorporation of L-lysine- $U-C^{14}$ into testis slices at 26, 32, and $37.5^\circ C$, at time points ranging from 15 minutes to 120 minutes, is shown in Figure 5. The results show that as the time of incubation is increased, the incorporation of isotope becomes progressively less at $37.5^\circ C$ than at $32^\circ C$. This indicates an increasing inhibition of the protein synthesizing system of the testis by the temperature afforded by the abdomen.

E. VIABILITY OF RAT TESTIS SLICES

In order to determine the viability of rat testis slices incubated for one hour at $37.5^\circ C$, studies were carried out employing D-glucose- $U-C^{14}$ as a labeled precursor for the biosynthesis of CO_2 and glutamic acid (Figure 6). Radioactivity in these substances was determined as a measure of the oxidative metabolism of the slice following incubation for one hour at 26.

32, and 37.5°C under an atmosphere of 95 per cent O₂ and 5 per cent CO₂. The amount of isotope transferred from labeled glucose to CO₂ in slices of rat testes incubated at these temperatures was 2,260; 2,840; and 3,360 counts/minute per 100 mg tissue, respectively. In a similar manner, the amount of isotope transferred from labeled glucose to glutamic acid in slices of testes incubated at 26, 32, and 37.5°C was 7,735; 8,030; and 9,240 counts/minute per 100 mg tissue, respectively. These data indicate that slices of rat testis are still viable after a one-hour incubation period at 37.5°C and that the possibility exists that the enzyme systems for protein biosynthesis in the testis may be selectively affected by an increase in temperature above 32°C.

F. EFFECT OF ABDOMINAL TRANSPLANTATION ON TESTICULAR WEIGHT AND INCORPORATION OF L-LYSINE-U-C¹⁴ INTO PROTEIN

1. Testicular weight.

The changes in the weight of testes subject to abdominal transplantation are indicated in Figure 7. A progressive decrease in the weight of the cryptorchid testis occurred up to 20 days following the surgical procedure, after which the weight of the cryptorchid testis reached a constant value. The scrotal testis, however, did not change appreciably in weight during this period.

2. Lysine incorporation.

Figure 7 also presents the incorporation of L-lysine-U-C¹⁴ into protein of slices of scrotal and cryptorchid testes at various time periods after surgery. Incubation of both scrotal

and cryptorchid testes was carried out at 32°C, since maximal incorporation had been observed to take place at this temperature. Following abdominal transplantation, the uptake of labeled lysine into protein per 100 mg wet weight of slices of the cryptorchid testis was found to gradually increase to approximately four times that which was observed for comparable slices of the scrotal testis. Maximal incorporation of L-lysine-U-C¹⁴ into proteins of slices of the cryptorchid testis occurred 20 days after abdominal transplantation.

G. EFFECT OF AGE AND ABDOMINAL CONFINEMENT ON THE SPECIFIC ACTIVITY OF RAT TESTIS SLICES

1. Effect of age on specific activity.

Figure 8 presents the effect of age on the incorporation of L-lysine-U-C¹⁴ into rat testis slices. A continual decrease in the specific activity (cpm/mg dry weight protein) of the scrotal testes was observed from 15 to 60 days of age, after which a constant value was reached. The specific activity fell from approximately 3,700 cpm to 300 cpm during this 35 day period.

2. Abdominal confinement.

Figure 8 also presents the effect of various lengths of exposure to the abdominal environment following transplantation of the right scrotal testis. From zero to twenty days the specific activity becomes constant to the farthest time point of forty days.

H. EFFECT OF TEMPERATURE ON THE UPTAKE OF LABELED LYSINE IN THE ADULT, SCROTAL TESTIS, THE CRYPTORCHID TESTIS, IMMATURE TESTIS, AND AN ANDROGEN-PRODUCING INTERSTITIAL TUMOR

Figure 9 is a comparison of the effect of temperature on the incorporation of L-lysine-U-C¹⁴ into protein of slices of the adult, scrotal testis, the cryptorchid testis 20 days after abdominal transplantation, the immature testis obtained from animals which were 20 days of age, and an androgen-producing, interstitial-cell testicular tumor. An increase in incubation temperature from 32 to 37.5°C resulted in a decrease in the uptake of labeled lysine into protein of slices of adult, scrotal testes. However, in the case of the cryptorchid testis, the immature testis and the interstitial-cell testicular tumor, protein labeling was found to increase with an elevated temperature of incubation.

I. HISTOLOGICAL COMPARISON OF SCROTAL TESTIS, CRYPTORCHID TESTIS, IMMATURE TESTIS, AND INTERSTITIAL-CELL TUMOR

1. Adult scrotal testis.

Figure 10 displays the histological picture of an adult scrotal testis. A normal basement membrane is seen consisting of spermatogonia and Sertoli cells. The more mature cell types, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa are also visible.

2. Cryptorchid testis.

~~The histological appearance of the cryptorchid testis 20~~

days following abdominal transplantation is also shown in Figure 10; beginning about 5 days after surgery, large numbers of degenerating spermatogenic cells were seen in the seminiferous tubular lumen. Vacuolation of cells with a decrease in maturing spermatids was a prominent feature. These atrophic changes progressed in severity, and at 20 days following abdominal transplantation, the only cellular elements of the seminiferous tubules found were large numbers of primary spermatocytes, some Sertoli cells, and a small number of spermatogonia. An increase in the total number of interstitial cells was not apparent, although these cells did appear to be confined to smaller areas because of the shrinkage of the seminiferous tubules.

3. Immature testis.

Histological examination of the immature rat testis obtained from animals which were 20 days of age revealed the presence of large numbers of primary spermatocytes with only occasional spermatogonia and Sertoli cells. The interstitial cells of Leydig were found to be completely absent in the immature rat testis at this age.

4. Androgen-producing interstitial-cell testicular tumor.

Histological examination of the androgenic interstitial-cell testicular tumor maintained in serial transplantation in BALB/c male mice indicated that this tissue consisted mainly of large vacuolated interstitial cells with some distended sinusoids and a few brown cells.

J. PROTEIN CONTENT OF 15,000xG SUPERNATANTS OF SCROTAL AND CRYPTORCHID TESTES UTILIZING THE LOWRY METHOD

Table 1 presents the results of four total protein determinations of 15,000xG supernatant fractions, containing ribosomes and pH5 fraction, from host scrotal and cryptorchid rat testes 30 days following abdominal transplantation. The average protein content of the scrotal testis was 51.9 mg/testis. The mean value for the cryptorchid testis was 14.5 mg/testis. Compared on a milligram per gram wet weight basis, the scrotal testis was found to contain 36.3 mg/gm wet weight tissue while the 30 day cryptorchid testis possessed 53.5 mg/gm wet weight tissue.

K. PROTEIN CONTENT IN WHOLE HOMOGENATES OF HOST SCROTAL AND CRYPTORCHID RAT TESTES UTILIZING THE LOWRY METHOD

Measurements of whole homogenates were made on 30 day abdominally confined testes and host scrotal testes. The data obtained is presented in Table 2. The average results for the scrotal testes and cryptorchid testes were 156.2 mg/testis and 30.0 mg/testis, respectively. The values adjusted to a mg/gm wet weight tissue ratio were 102.8 mg/gm wet weight tissue for the scrotal testis and 108.3 mg/gm wet weight tissue for the cryptorchid testis. The protein content can be calculated to represent 10.3 per cent of the scrotal testis and 9.9 per cent of the cryptorchid testis. These values are in good accord with those of Wolf (84) who found immature rat testes to contain approximately 10.5 per cent protein.

L. MOISTURE CONTENT OF HOST SCROTAL AND CRYPTORCHID RAT TESTES

Because of the dramatic weight loss observed in the cryptorchid testis, the role of fluid shifts was investigated as a possible explanation for this loss. Table 3 indicates that although the cryptorchid testis undergoes severe atrophy following 30 days in the abdomen, it still possesses approximately the same percentage of water as the scrotal, 83.7 as compared to 87.1. This would seem to rule out tissue fluid loss as the cause of the decrease in size.

CHAPTER IV

DISCUSSION

The testicular atrophy which results in testes confined in the abdomen has been attributed to the higher temperature of this region over that found in the scrotal compartment. That the testis should be so selectively sensitive to this physiological environment suggests some departure or eccentricity in its metabolic constitution. Since the temperature phenomenon is believed to be the critical entity involved, it became of interest to study this factor in relation to protein synthesis. The synthesis of proteins was chosen because they represent the most heat sensitive constituents of the cell, and, therefore, the area in which damage caused by heat would be manifested first. The albino rat was chosen as the experimental animal because of its availability and ease in handling. Its testes are large enough to provide ample tissue for protein incorporation studies.

The first concern, prior to studying the protein synthesizing system itself, was to determine the temperature gradient which exists between the peritoneal cavity and the scrotum in the rat. Results obtained showed an approximate 3°C difference. This substantiated the values reported by other investigators (86). In view of this confirming evidence, it was then plausible to continue the line of investigation.

L-lysine-U-C¹⁴ was chosen as the label because this amino acid is known to be almost entirely utilized in the synthesis of proteins, its involvement in other metabolic sequences being minimal. Kinetic studies were carried out on the incorporation of L-lysine-U-C¹⁴ into protein of rat testis slices in order to determine the time of incubation wherein the isotope uptake remained linear while still possessing a specific activity (cpm/mg dry weight protein) well over background counting. A one-hour time period was chosen. On the basis of a one-hour incubation period, it became necessary to determine whether the tissue remained viable after this length of time. Oxidative metabolism was chosen as the criterion of viability, and the radioactivity of glutamate and CO₂ upon incubation with D-glucose-U-C¹⁴ was used as respiratory indicators. Both glutamate and CO₂ exhibited a linear response to the temperatures during their one-hour incubation period, thus indicating a responsive system.

The incorporation of L-lysine-U-C¹⁴ was then studied in slices of testis, liver, kidney, and spleen at temperatures ranging from 20 to 37.5°C. This was done in order to compare the testis with tissues that normally operate at the temperature of the abdominal cavity. Biological variation was minimized by utilizing three temperature controlled water baths, which allowed the simultaneous incubation of tissues excised from one animal. The results showed a progressive increase in isotope uptake from 20 to 32°C in all four tissues. From 32 to 37.5° C, however, the

specific activity of the testis fell to approximately one-half of its value at 32°C. Liver, kidney, and spleen continued to reflect an increase in protein synthesis from 32 to 37.5°C. This experiment demonstrated that the temperature of the peritoneal cavity, to which the liver, kidney, and spleen are normally exposed, was detrimental to the protein synthesizing system of the testis. This was the first substantial evidence obtained in this study which suggested a heat labile mechanism to be operable in the rat testis.

In view of the observed temperature effect on the apparatus involved in lysine uptake, it became of interest to study the effect of incubating testis slices at 26, 32, and 37.5°C for time periods ranging from 15 minutes to 2 hours and determining lysine incorporation as the specific activity. If 37.5°C represents an unphysiological temperature for the testis, then this effect should be magnified over a longer incubation period. The results obtained reflected a progressive decrease in protein synthesis at the elevated temperature as the time of incubation was lengthened. This may indicate the involvement of a protein denaturing effect, since as the time of exposure to the higher temperature is increased, there exists a greater difference in specific activity between 32 and 37.5°C. Results such as these would be expected if a thermolabile system were present, for as the time of exposure to the higher temperature increases, a greater quantity of the protein would be adversely affected.

The previous "acute" in vitro experiments suggested the study of chronically prepared cryptorchid animals as an avenue of investigation. First, however, the incorporation of L-lysine-U- C^{14} was studied in normal animals of different age. Sixty day old rats were ultimately selected since they possess, histologically and morphologically, a mature adult testis, and since the specific activity was found to be sufficiently above background. The testis was allowed to remain in the abdominal cavity up to 40 days. At time points ranging from 5 to 40 days it was removed and slices incubated at 32°C for one hour. As the time of abdominal confinement increased, there was a parallel increase in specific activity up to 20 days following abdominal transplantation at which point a constant level was reached. The scrotal testis was found to have no change in its specific activity. The increase in the specific activity of the cryptorchid testis seems paradoxical in view of its dramatic reduction in weight. A survey of the literature, however, revealed that the various cellular components of the testis, germinal cells, Sertoli cells, and Leydig cells possess independent metabolic characteristics (74, 76, 77). The overall metabolism of the testis, therefore, represents an integration of these distinct influences. The data of the present studies indicate that experimental cryptorchidism in the rat is characterized by a marked increase in the incorporation of L-lysine-U- C^{14} into testicular protein. The predominant histological feature of the cryptorchid testis is the absence of

virtually all of the spermatids and mature sperm. However, the spermatogonia, primary spermatocytes, Sertoli cells, and the interstitial cells of Leydig appear to resist degeneration. The Sertoli cells do undergo some transformations, however, in that between the 10th and 21st post-operative day there is an increase in the number of nuclei. This increase is accompanied by a significant decrease in nuclear size; it is considered that these changes may be the result of amitotic division (17). The possibility therefore exists that the increased level of protein labeling which was observed in the cryptorchid testis is due to the persistence of some of these latter cell types.

If, in fact, cell types do influence the specific activity, then an investigation of various testicular states, in which the cellular constitution differed, would be expected to show variation. Slices of adult, scrotal testes, cryptorchid testes, immature testes, and an androgen producing testicular tumor were incubated for one hour at 32 and 37.5°C. As expected, the scrotal testicular tissue showed a decrease in specific activity between 32 and 37.5°C. However, in the case of the cryptorchid testis, immature testis, and the androgen producing testicular tumor, there was an increase in the specific activity. Histological comparison of all four tissues revealed that only in the adult, scrotal testis did mature sperm and spermatids constitute a relatively large proportion of the organ. This suggested that these two cellular forms were the most heat labile and that their

absence resulted in an "unmasking" of the metabolic characteristics of the remaining cells. Inasmuch as the immature testis consists essentially of primary spermatocytes with only occasional spermatogonia and Sertoli cells and a total absence of Leydig cells (50), it would appear that the Leydig cells are not responsible for the increased uptake of labeled lysine into protein of slices of the cryptorchid testis. What is more likely is that the cryptorchid testis, following the disappearance of other cell types, reflects the metabolism of the more primitive and highly undifferentiated spermatogonia and primary spermatocytes and that these are the cell types of the testis that have the highest degree of protein labeling from radioactive lysine.

That the various cellular groups of the testis possess diversified metabolism and that their relative proportion can influence the overall metabolism observed in the testis has been illustrated by several investigators. Perlman (66) found an increase in the concentration of cholesterol in the cryptorchid testis and suggested that the spermatogonia, primary spermatocytes, and possibly the Sertoli cells contain the highest cholesterol concentrations. Lynch (42) also reports an accumulation of lipids near the basement membrane in cases of artificial cryptorchidism. Steinberger (72, 73) has studied the histological picture of the testis in regards to hyaluronidase production. He cites a correlation between the presence of spermatids and the concentration of hyaluronidase. These last few examples

illustrate the influence of the various testicular components on the metabolic expressions of the organ.

Although the increased incorporation of labeled lysine into protein of the testes of young rats may be accounted for by a higher concentration of ribonucleic acid (RNA) (50), no such increase in RNA has been found in samples of cryptorchid testes (51).

The differentiation of the immature cell types into tubular spermatozoa apparently involves some process whereby the mature cell forms become more heat labile to the effect of heat and more susceptible to substrate deficiency. It is interesting to note that these cells most adversely affected by temperature are those involved in meiotic transformations. A correlation between a thermo-labile meiotic apparatus and spermatogenic failure might be possible. Additional studies are planned in this regard. Whatever alteration in heat sensitivity is brought about in the rat tubular spermatozoa, it is apparently not carried over to ejaculated forms. Beck (7) reports that the maximum metabolic activity of warm blooded vertebrate spermatozoa occurs at about 40 to 45°C, and that temperatures in the range of 48 to 55°C cause death. The values quoted for maximum activity are obviously in excess of the abdominal temperature which causes atrophy of the rat testis.

In view of the atrophy of the testis when retained in the abdomen and its elevated specific activity, it was decided to measure total protein concentration in whole homogenates and

15,000xG supernatants (containing ribosomes and soluble fraction). In the series of experiments on whole homogenates the cryptorchid testes were found to weigh 1/5 that of their scrotal mates. The concentration of protein (mg protein/testis) was found to parallel this ratio in that the cryptorchid testis contained 1/5 the amount of the normal. Compared on a mg/gm wet weight tissue basis, there was no significant difference. This data infers that there is no overall net stimulation of protein synthesis in the cryptorchid testis.

Since fluid shifts represent the most mobile mass in any tissue, it was conceivable that in the cryptorchid testis there might exist a dehydration state, and thus account for the great loss in cellular weight. Determination of moisture content was thereby singularly important because this commodity constitutes the greatest proportion in any tissue. Moisture measurements were made on both the normal and cryptorchid testis. An approximately equivalent amount was found in both cases. The results obtained rule out fluid loss as the cause of testicular atrophy.

The exact mechanism of the damaging effect of temperature on the germinal epithelium is as yet undetermined. The possibility of enzyme inactivation or deficiencies in substrate, brought about by vascular changes, appear to be the most promising areas of investigation. Of the data accumulated so far, the most pertinent to this research is the influence of the different cell types of the testis on its overall metabolism. Histological changes have

been correlated with altered metabolism (76). This is very important, for, in effect, comparing the normal testis with the cryptorchid testis is similar to comparing two different tissues which happen to possess some morphological characteristics in common. These data suggest that while the spermatogonia and primary spermatocytes display the highest degree of protein labeling from radioactive lysine, protein labeling from radioactive lysine in the spermatids shows the greatest susceptibility to elevated temperature.

CHAPTER V

SUMMARY

1. Measurement of the peritoneal and scrotal temperatures of the adult white rat demonstrated that the scrotal temperature was approximately three degrees lower than that of the peritoneal cavity.

2. A study of the time course of L-lysine-U-C¹⁴ incorporation into protein of adult rat testis slices was made, and one-hour was chosen as the time of incubation for further studies.

3. A comparison of the uptake of labeled lysine was made with testis, liver, kidney, and spleen at 20, 26, 32, and 37.5°C. Increase in specific activity was noted for all tissues up to and including 32°C. However, whereas the liver, kidney, and spleen continued to increase from 32 to 37.5°C, the testis showed a decrease.

4. Comparison of scrotal and cryptorchid testis weights were made following various lengths of time in the abdominal cavity. The scrotal testis was found to maintain a rather constant weight. The cryptorchid testis, however, decreased in size until it stabilized in weight after 20 days in the abdomen.

5. Comparison of total cpm in protein per 100 mg wet weight testis slice of the scrotal and cryptorchid testis was determined following an in vitro incubation with L-lysine-U-C¹⁴. The values

for the scrotal testis remained constant to the farthest time point studied. On the other hand, the uptake of the cryptorchid testis steadily increased to four times that observed for the scrotal testis twenty days following abdominal transplantation.

6. The effect of age on the incorporation of L-lysine- $U-C^{14}$ into protein of rat testis slices was investigated. The specific activity (cpm/mg protein) dropped from a high of 3,700 cpm at 15 days of age to a low of 300 cpm at 60 days of age, at which point it became constant.

7. The data obtained indicate that the enzymatic systems involved in the incorporation of labeled lysine into proteins of the rat testis are more heat labile than those of rat liver, kidney and spleen. It is suggested that the increased heat lability of the protein synthesizing system of the testis may offer a partial explanation for the deleterious effects of temperature on spermatogenesis occurring in cases of cryptorchidism.

8. An attempt has been made to correlate the effects of temperature on protein labeling of various cell types of the rat testis. An elevated temperature of incubation was found to decrease testicular protein labeling from radioactive lysine in slices of adult scrotal testes but not in slices of the cryptorchid testis, the immature testis or an interstitial-cell testicular tumor consisting of only Leydig cells. The data obtained indicate that while the spermatogonia and primary spermatocytes display the highest degree of protein labeling from radioactive

lysine, protein labeling in the spermatids shows the greatest susceptibility to elevated temperatures.

CHAPTER VI

FIGURES

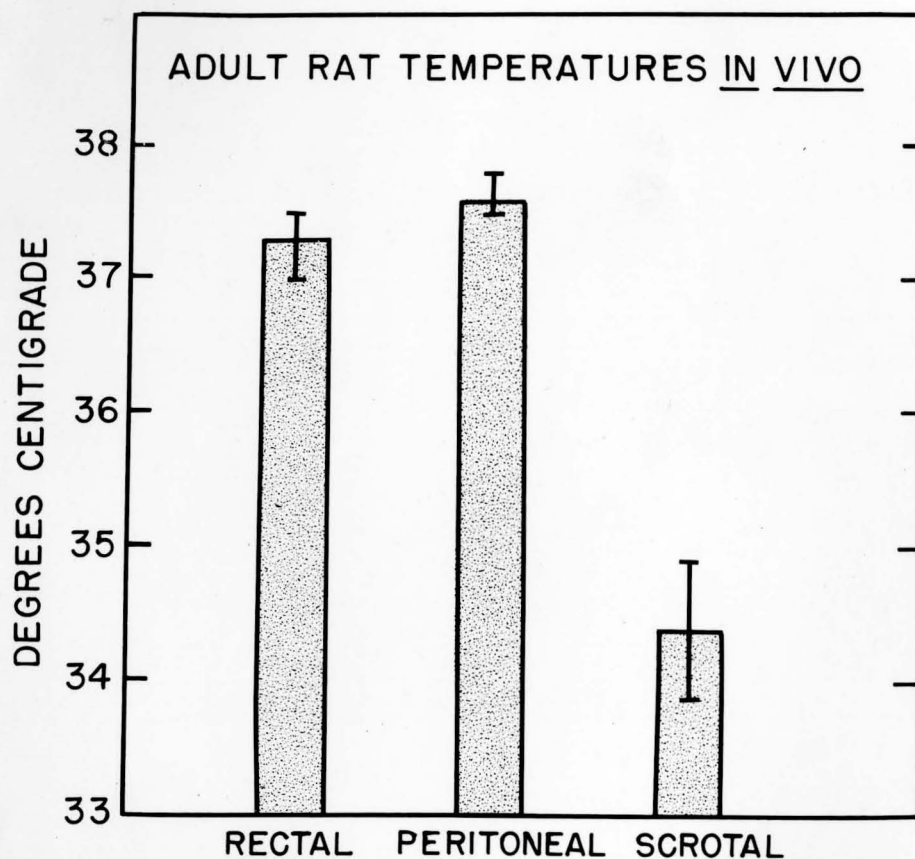


FIGURE I

In vivo temperature measurements were made of rectal, peritoneal and scrotal regions of the adult rat. Recordings were obtained by utilizing thermo-couple probes at room temperature. The ranges of rectal, peritoneal and scrotal temperatures were 37-37.5, 37.4-37.8 and 33.7-34.9°C, respectively.

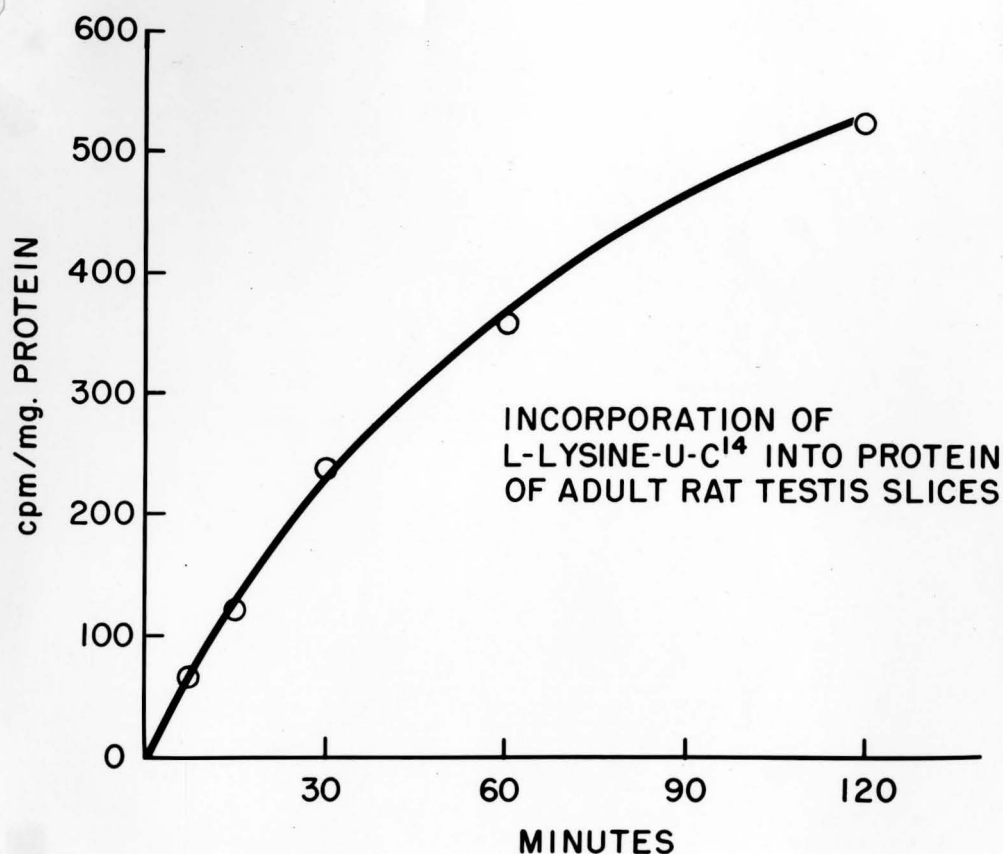


FIGURE II

Incorporation of L-lysine-U-C¹⁴ into protein of slices of rat testis. The flasks contained 250,000 counts/minute of L-lysine-U-C¹⁴ and Krebs-Ringer bicarbonate buffer (pH 7.4) in a total volume of 3.2 ml. The gas phase was 95 per cent O₂ and 5 per cent CO₂. The incubation temperature was 37.5°C. Values are averages of three experiments.

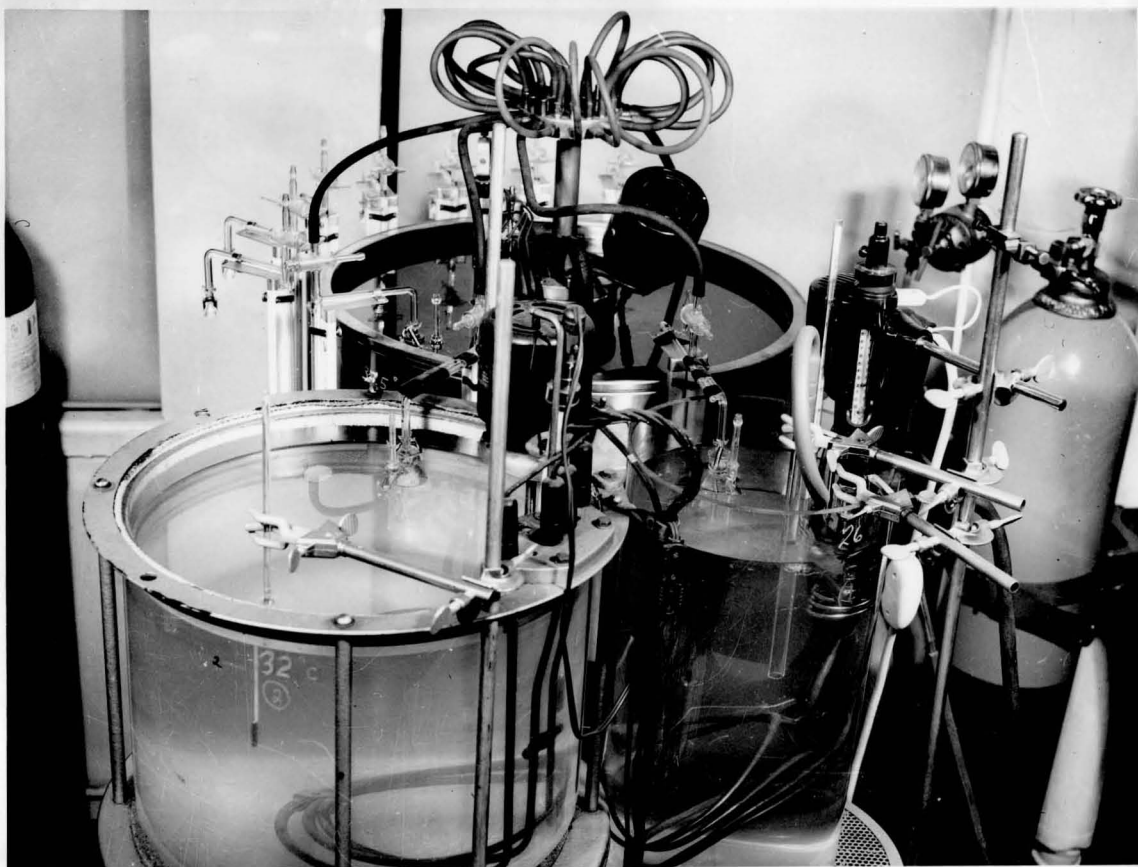


FIGURE III

The incubation device employed in these experiments is shown above. It is composed of three temperature-controlled water baths surrounding a main Warburg apparatus. The manometers were arranged so that each flask was allowed to shake at 140 oscillations/minute at the desired temperature during the incubation period.

EFFECT OF TEMPERATURE ON INCORPORATION OF
L-LYSINE-U-C¹⁴ INTO PROTEIN OF RAT TISSUE SLICES

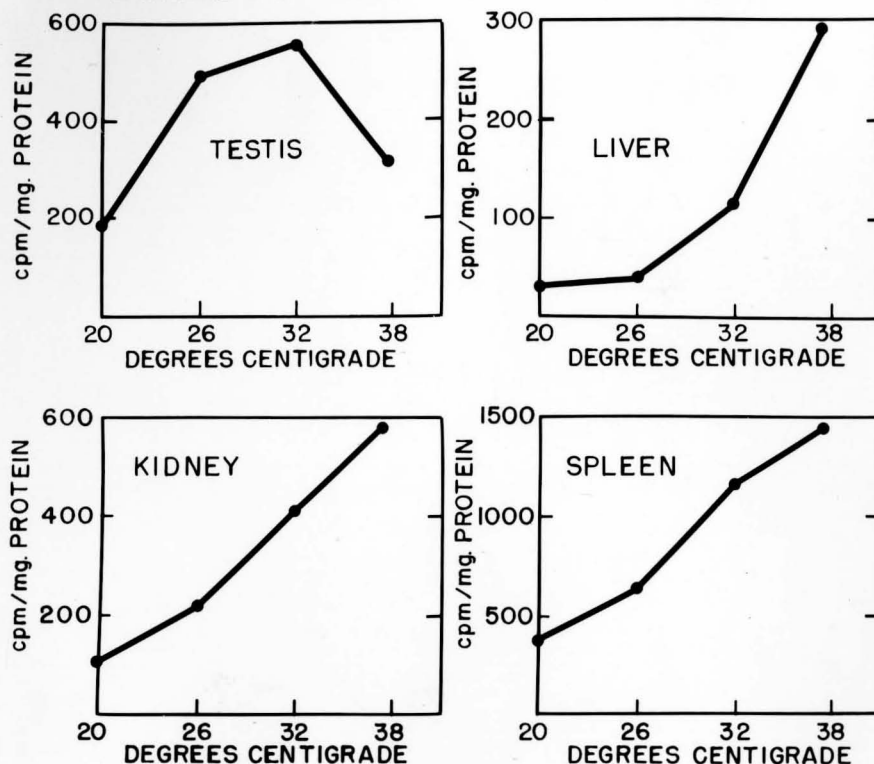


FIGURE IV

Effect of temperature on the incorporation of L-lysine-U-C¹⁴ into protein of slices of rat testis, liver, kidney, and spleen. The time of the incubation was one hour. Each point on the curves represents the average of six experiments for samples of testis, five experiments for samples of liver, and four experiments for samples of kidney and spleen. The values, expressed as counts per minute per milligram protein \pm the standard error, obtained following incubation at 32 and 37.5°C, respectively, are as follows: for the testis, 562 ± 53 and 346 ± 19 ; for the liver, 119 ± 11 and 257 ± 33 ; for the kidney, 407 ± 58 and 581 ± 56 ; for the spleen, 116 ± 89 and 1435 ± 53 .

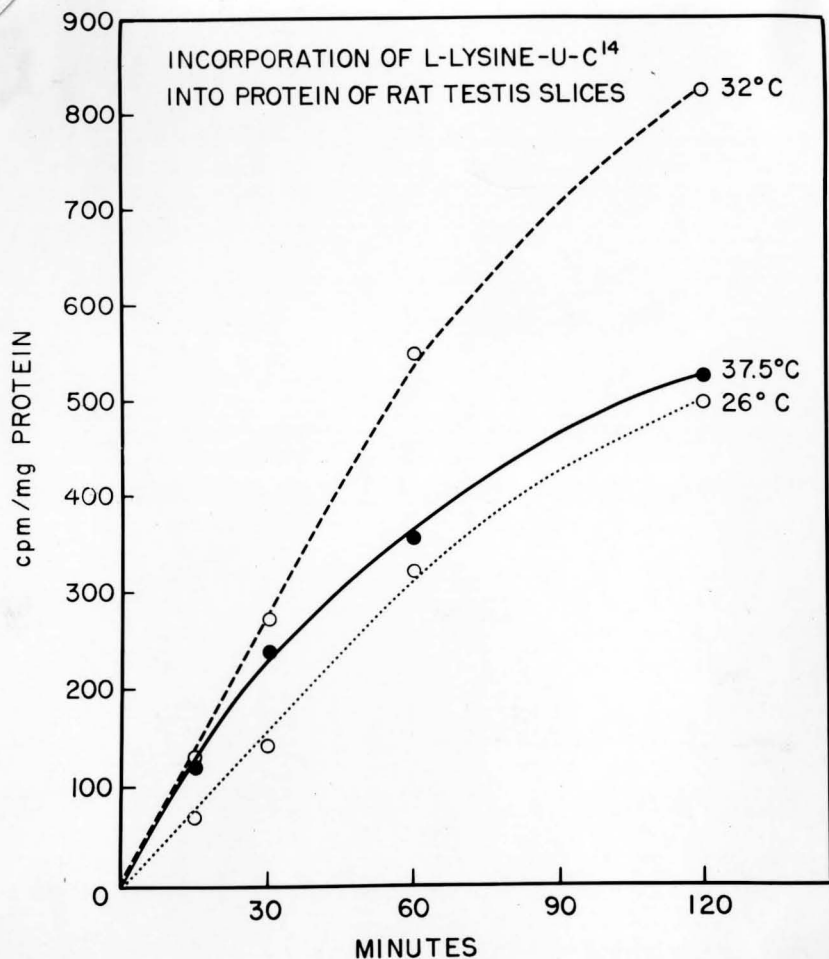


FIGURE V

Incorporation of L-lysine-U-C¹⁴ into protein of rat testis slices at 26, 32, and 37.5°C at various time intervals. Flasks containing tissue samples from the same animal were run concurrently thus eliminating a great deal of biological variation.

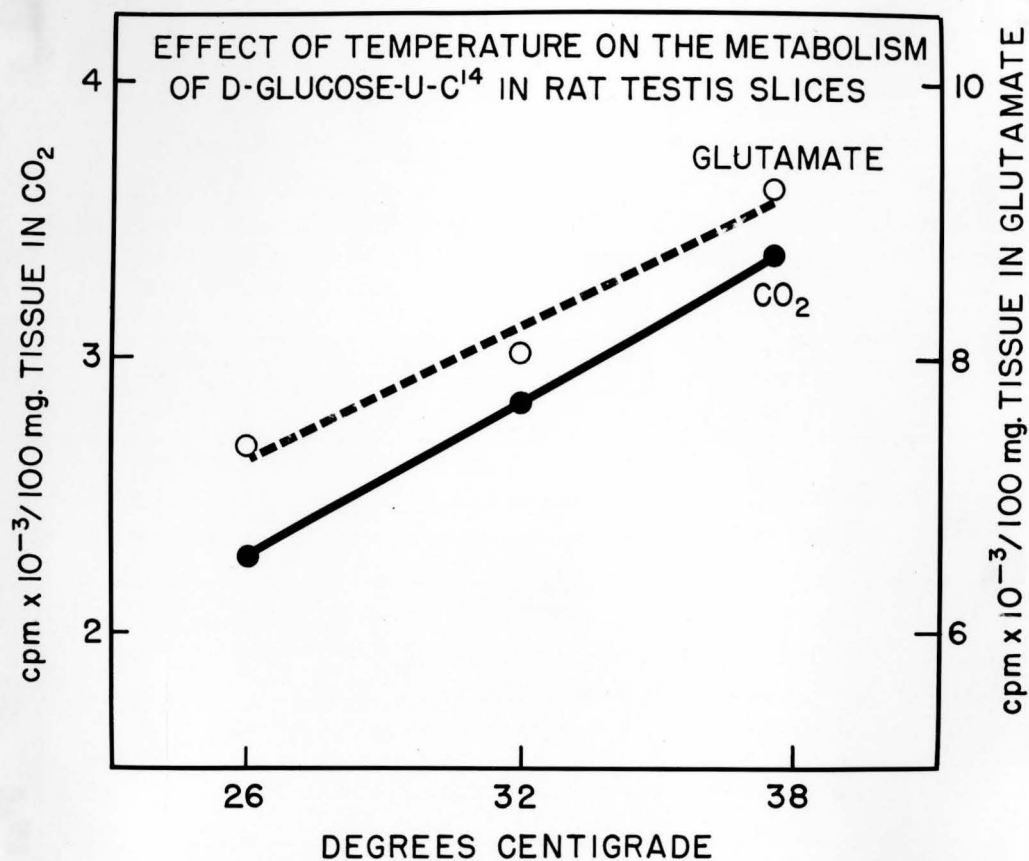


FIGURE VI

A viability study of rat testis slices incubated for one hour was carried out. Radioactivity in these slices was determined as a measure of the oxidative metabolism following incubation under an atmosphere of 95 per cent O₂ and 5 per cent CO₂.

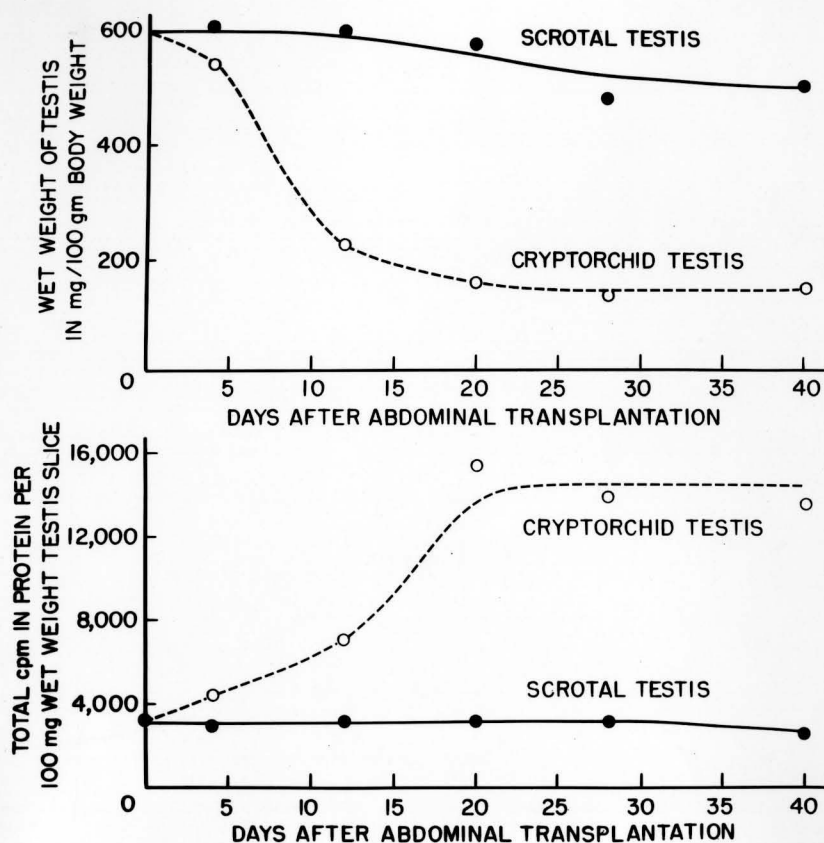


FIGURE VII

Effect of abdominal transplantation on testicular weight and incorporation of L-lysine- $U-C^{14}$ into protein. Flask contents: L-lysine- $U-C^{14}$, 250,000 cpm in 0.2 ml; Krebs-Ringer bicarbonate buffer, pH 7.4, to a total volume of 3.2 ml. The gas phase was 95 per cent O_2 and 5 per cent CO_2 . Flasks were incubated for one hour at $32^\circ C$. Each point on the curve represents the average of four experiments.

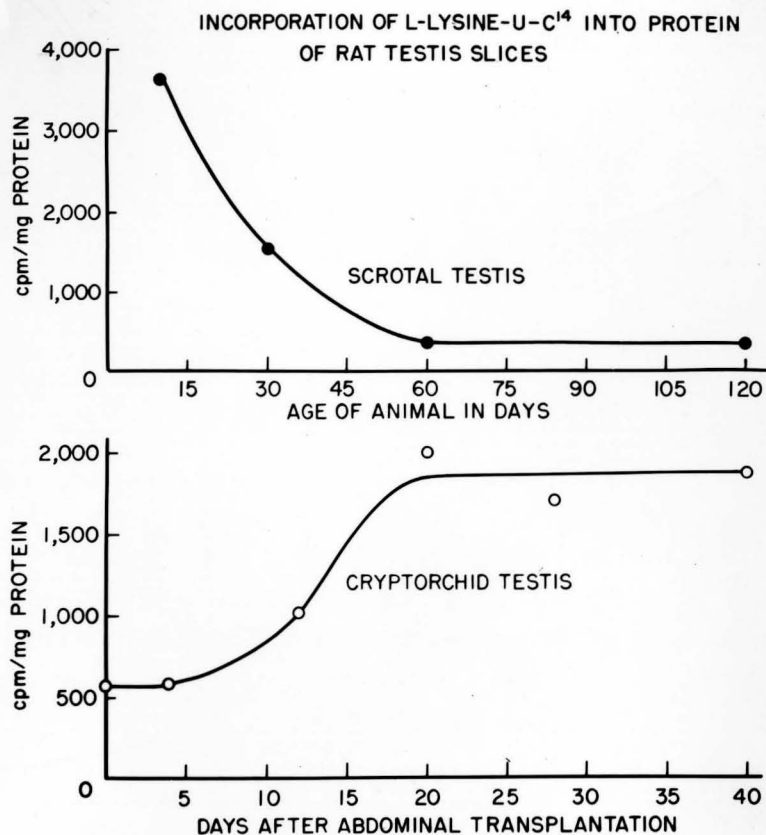


FIGURE VIII

The effect of animal age on L-lysine-U-C¹⁴ uptake into testis slices is represented by the upper graph. The lower graph illustrates the effect of varying lengths of abdominal confinement on the specific activity (cpm/mg protein) of the cryptorchid testis.

EFFECT OF TEMPERATURE ON THE INCORPORATION OF L-LYSINE-U-C¹⁴ INTO
PROTEIN OF TESTIS SLICES

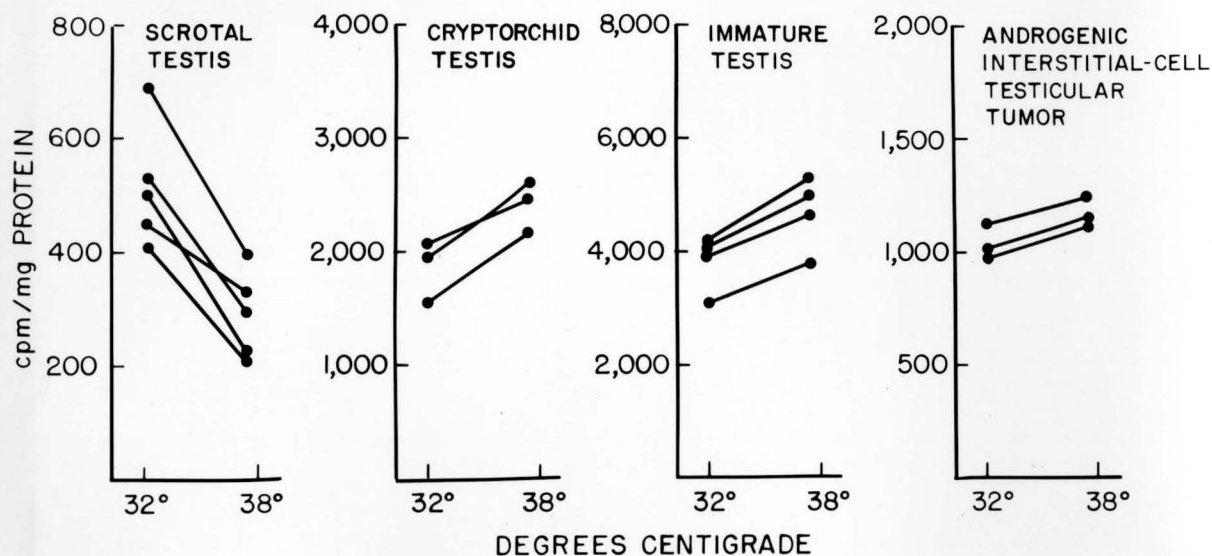
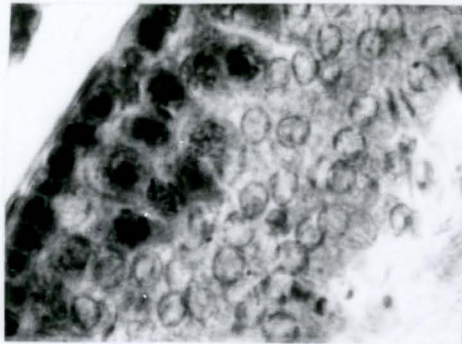
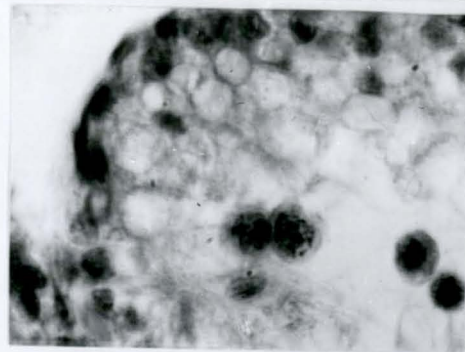


FIGURE IX

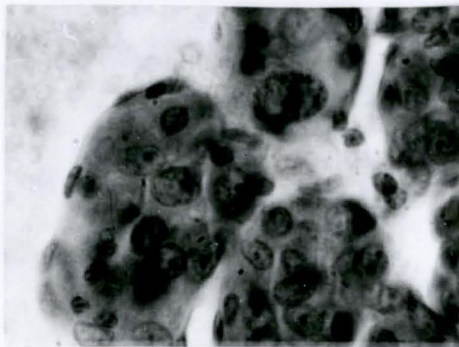
Effect of temperature on the incorporation of L-lysine-U-C¹⁴ into protein of slices of adult, scrotal testes, the cryptorchid testis 20 days after abdominal transplantation, the immature testis obtained from animals which were 20 days of age, and an androgen-producing interstitial-cell testicular tumor. Time of incubation was one hour. Each line represents tissue samples obtained from a single animal and incubated simultaneously at 32 and 37.5°C, respectively.



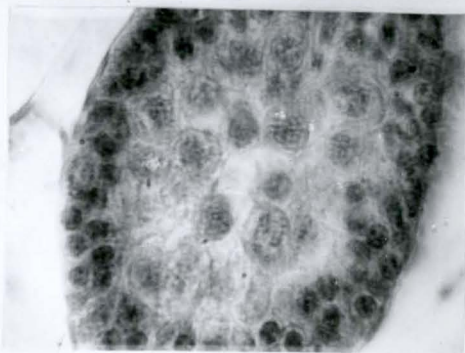
ADULT SCROTAL
TESTIS



CRYPTORCHID
TESTIS



INTERSTITIAL-
CELL
TESTICULAR



IMMATURE
TESTIS

FIGURE X

A histological comparison of the adult, scrotal testis; 20-day cryptorchid testis; interstitial-cell testicular tumor; and 20-day old immature testis. The tissues were stained with Hematoxylin-eosin and photographed at a magnification of 700X.

CHAPTER VII

TABLES

TABLE I

PROTEIN CONTENT IN 15,000xG SUPERNATANTS
CONTAINING RIBOSOMES AND SOLUBLE FRACTION OF HOST SCROTAL AND
CRYPTORCHID RAT TESTES 30 DAYS AFTER ABDOMINAL TRANSPLANTATION

	Total Wet Weight of Testis (gm)	FOLIN REAGENT	
		Mg/Testis	Mg/gm Wet Weight Tissue
Scrotal Testis	1.582 } 1.412 } 1.428 1.431 } 1.288 }	57.5 } 51.2 } 51.9 48.4 } 50.4 }	36.3 } 36.1 } 36.3 33.8 } 39.1 }
Crypt. Testis	0.297 } 0.259 } 0.271 0.287 } 0.241 }	16.1 } 14.2 } 14.5 14.5 } 13.4 }	53.1 } 54.8 } 53.5 50.2 } 55.7 }

TABLE II

PROTEIN CONTENT IN WHOLE HOMOGENATES OF HOST SCROTAL AND
CRYPTORCHID RAT TESTES 30 DAYS AFTER ABDOMINAL TRANSPLANTATION

	Total Wet Weight of Testis (gm)	Folin Reagent	
		Mg/Testis	Mg/gm Wet Weight Tissue
Scrotal Testis	1.499 } 1.572 } 1.520 1.489 } 1.522 }	110.0 } 171.6 } 156.2 187.1 } 131.0 }	73.5 } 109.2 } 102.8 125.6 } 86.1 }
Crypt. Testis	0.359 } 0.264 } 0.304 0.288 } 0.266 }	23.2 } 29.9 } 30.0 37.0 } 24.0 }	83.2 } 113.0 } 108.3 128.6 } 90.4 }

TABLE III

MOISTURE CONTENT OF HOST SCROTAL AND
CRYPTORCHID RAT TESTIS 30 DAYS AFTER ABDOMINAL TRANSPLANTATION

	Total Wet Weight of Testis (gm)	Per Cent H ₂ O
Scrotal Testis	1.902 } 1.516 } 1.662 1.567 }	87.3 } 86.9 } 87.1 87.1 }
Cryptorchid Testis	0.413 } 0.351 } .378 0.369 }	81.6 } 84.7 } 83.7 84.8 }

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APPROVAL SHEET

The thesis submitted by Mannfred A. Hollinger has been read and approved by three members of the faculty of Loyola University.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

Jan. 11, 1965
Date

Joseph R. Davis, M.D., Ph.D.
Signature of Advisor